Optimal length of cultivation time for isolation of propionibacterium acnes in suspected bone and joint infections is more than 7 days

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Abstract: Diagnosis of Propionibacterium acnes bone and joint infection is challenging due to long cultivation time of up to 14 days. We retrospectively studied whether reducing cultivation time to 7 days allows accurate diagnosis without losing sensitivity. We identified patients with at least one positive P. acnes sample between 2005 and 2015 and grouped into 'infection' and 'no infection'. An 'infection' was defined when at least two samples from the same case were positive. Clinical and microbiological data including time to positivity for different cultivation methods were recorded. We found 70 cases of proven P. acnes infection with a significant faster median time to positivity of 6 days (range 2-11 days) compared to 9 days in 47 cases with P. acnes identified as a contamination ($p<0.0001$). In 15 of 70 (21.4%) patients with an infection, tissue samples were positive after day 7 and in 6 patients (8.6%) after day 10 when performing a blind subculture of the thioglycolate broth. Highest sensitivity was detected for thioglycolate broth (66.3%) and best positive predictive values for anaerobic agar plates (96.5%). A prolonged transportation time from the operating theatre to the microbiological laboratory did not influence time to positivity of P. acnes growth. By reducing the cultivation time to 7 days, false negative diagnosis would increase by 21.4%, thus we recommend to cultivate biopsies in bone and joint infections to detect P. acnes for 10 days with a blind subculture at the end.

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Optimal cultivation length to isolate *Propionibacterium acnes* in suspected bone and joint infections is > 7 days

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Running Head: Time to positivity in *Propionibacterium acnes*

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ABSTRACT

Diagnosis of *Propionibacterium acnes* bone and joint infection is challenging due to long cultivation time of up to 14 days. We retrospectively studied whether reducing cultivation time to 7 days allows accurate diagnosis without losing sensitivity. We identified patients with at least one positive *P. acnes* sample between 2005 and 2015 and grouped into 'infection' and 'no infection'. An 'infection' was defined when at least two samples from the same case were positive. Clinical and microbiological data including time to positivity for different cultivation methods were recorded. We found 70 cases of proven *P. acnes* infection with a significant faster median time to positivity of 6 days (range 2-11 days) compared to 9 days in 47 cases with *P. acnes* identified as a contamination ($p<0.0001$). In 15 of 70 (21.4%) patients with an infection, tissue samples were positive after day 7 and in 6 patients (8.6%) after day 10 when performing a blind subculture of the thioglycolate broth. Highest sensitivity was detected for thioglycolate broth (66.3%) and best positive predictive values for anaerobic agar plates (96.5%). A prolonged transportation time from the operating theatre to the microbiological laboratory did not influence time to positivity of *P. acnes* growth. By reducing the cultivation time to 7 days, false negative diagnosis would increase by 21.4%, thus we recommend to cultivate biopsies in bone and joint infections to detect *P. acnes* for 10 days with a blind subculture at the end.

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INTRODUCTION

Propionibacterium acnes is a facultative anaerobic Gram-positive rod, abundant on the human skin, and mainly associated with sebaceous glands of the shoulder and axilla (1). It is most commonly associated with the chronic skin disease acne vulgaris. However, it may also cause bone and joint infections including implant-associated infections. *P. acnes* has been recognized as an emerging cause of shoulder infections (2, 3) and is among the most common pathogens isolated in shoulder periprosthetic joint infections (PJI) (4, 5). *P. acnes* has also been implicated in other biofilm-related infections (6-8) such as cardiovascular implant-associated infections (9), spinal osteomyelitis (10, 11), and endophthalmitis (12, 13).

Diagnosis of *P. acnes* bone and joint infections is challenging since pain is often the only symptom (14, 15). For long-time, *P. acnes* was underdiagnosed in bone and joint infections due to the short cultivation time routinely used in diagnostic laboratories. In general, biofilm forming bacteria are known to replicate at a slow rate due to low metabolism (16). Since recent studies recommended a prolonged cultivation time of up to 14 days for bone and joint infections (17, 18), the diagnosis of *P. acnes* infections has become more frequently documented (19). In view of the high costs of a long incubation period, a recent study suggested that 7 days of incubation should be sufficient for accurately diagnosing orthopedic implant-associated infections (20). In this study, 96.6% of the infections were detected within 7 days. However, *P. acnes* caused only one out of the 58 infections. The majority of cases was predominantly caused by *Staphylococcus aureus* and coagulase-negative staphylococci. Other studies attempted to improve diagnostic quality using better cultivation methods or sonication of implants

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to improve sensitivity and expedite diagnosis (21-24). However, none of these studies included a sufficient number of *P. acnes* infections to perform statistical analysis, which would allow drawing conclusions about the ideal cultivation time for slow-growing microorganisms such as *P. acnes*.

This study reports the results of a large cohort of patients with *P. acnes* positive samples, analyzing and comparing time to detection in bone and tissue biopsies, and of sonicated implants with detailed information about transportation time, media types, and handling of specimens. We hypothesized that a higher inoculum as found in bone and joint infections would show growth of *P. acnes* earlier than a lower inoculum due to contamination. Therefore we compared time to positivity in patients with an infection caused by *P. acnes* and isolation of *P. acnes* interpreted as a contaminant. The main aim of the study was to answer the question whether reducing the cultivation time to 7 days allows to diagnose *P. acnes*-related bone and joint infections without losing sensitivity.

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METHODS

Study population

The orthopedic University Hospital Balgrist in Zurich, Switzerland is a 120-bed orthopedic center specialized for bone and joint infections. Approximately 5000 surgical procedures are performed annually thereof 326 primary hip, 186 knee, and 143 shoulder arthoplasties in 2015. In this single-center study, we retrospectively searched for patients with an orthopedic surgery (bone, or joint related) with at least one positive sample for *P. acnes* isolated between January 2005 and December 2015. All positive and negative samples within the same patient, same hospitalization period, and same infection site were grouped as a diagnostic set in a patient case.

Microbiological data were searched using the database of the Institute of Medical Microbiology, University of Zurich, Zurich, Switzerland. Clinical and demographic parameters of the patients' medical history at the time of diagnostic work-up including diagnosis were investigated using the patient clinical database of the orthopedic department and the prospective database of the Infectious Diseases Consultation service. We grouped patients into the following 2 groups: 'infection' with growth of *P. acnes* in 2 or more samples within the same case and 'no infection' with only 1 sample positive for *P. acnes* suggesting contamination of the sample. For optimal and accurate allocation in one of the two groups 'infection' and 'no infection', only cases with 3 or more samples were included for our analysis (25, 26). Although diagnosis of 'infection' was strictly microbiological defined, we also reviewed clinical presentation of all patients (presence of sinus tract) and investigated the intraoperative presentation. 'Infections' were further grouped as i) associated with a joint arthroplasty (PJI), ii) associated with

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another orthopedic implant than arthroplasty (implant-associated infections), iii) septic arthritis (often arthroscopy-associated), or as iv) osteomyelitis. Implants other than arthroplasties were labeled as small if screws or anchors were in place, and as large if plates or intramedullary nails were used. Intraoperative samples for microbiological diagnostics could be tissue or bone biopsies (peri- or intraarticular, periimplant) for conventional culture, and the implant itself for culture of sonication fluid in case of removal of the implant (27). In cases with septic arthritis or osteomyelitis only tissue or bone biopsies were investigated.

Synovial fluid samples were excluded because we assumed faster time to positivity from recovered planktonic bacteria than biofilm bacteria isolated from periimplant tissue or bone. Samples to analyze should have a minimal acceptable cultivation time in order not to distort statistical analysis. Consequentially we excluded negative samples with a too short cultivation time according to Table 1. Since antibiotic treatment could influence time to positivity of *P. acnes* growth, we excluded samples from patients taken antibiotics for ≥ 24hours within 14 days prior to sample acquisition. Furthermore, we excluded patients with polymicrobial infections since other bacteria in the same sample - in particular virulent bacteria, could decrease detection of *P. acnes*. All excluded samples and cases are numerated in the Supplementary Table S1. The study was performed in line with the current ethical guidelines and approved by the Institutional Review Board in Zurich, Switzerland.

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Analysis and statistical methods

For each sample of a patient diagnostic set, we recorded culture details such as type (e.g. tissue, bone, sonication fluid, synovial fluid, or wound swab), culture method, and Gram staining. We calculated time to positivity of each positive sample and culture type in the group of cases with an 'infection' and cases without.

Transportation time was calculated as the difference in hours between time of acquisition of samples during surgery and arrival at the microbiological laboratory. Since the exact time of biopsy acquisition had not been recorded in the medical chart, we defined time of acquisition as the mean in time between start and end time of surgery.

We analyzed time to positivity, sensitivity, specificity, and positive and negative predictive value for each culture method (direct aerobic, direct anaerobic, and enrichment) for tissue and bone biopsies, and sonication fluid from explanted hardware. To calculate diagnostic sensitivity, we compared test performance in patients with an 'infection' (number of positive samples divided by all samples taken within a case) and specificity in patients without (number of negative samples divided by all samples taken within a case).

Statistical analysis was conducted using Stata 14.1 SE (StataCorp College Station, Tx). We used non-parametric test-statistics: Wilcoxon rank-sum tests to compare continuous variables and Fisher's exact tests to compare categorical variables. To assess if transportation time from the operation theatre to the microbiological laboratory was correlated with culture time to positivity, we used the Spearman's rank correlation coefficient. Kaplan-Meier curves were used to illustrate the number of days from sampling to culture positivity. For patients belonging to the group of *P. acnes*

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'infections', time was measured until culture positivity of the second sample to confirm 'infection'. Differences between groups were analyzed with log-rank tests.

Microbiological processing

Cultures

Prediagnostic. Periimplant-tissue, explanted hardware (implant), and bone samples were transported from the operating room at the orthopedic hospital Balgrist to the microbiology laboratory, which is a 30 to 60 minutes' drive by car. Transport service for microbiological samples is twice daily. Tissue or bone samples were transported in culture tubes without adding transporting media, implants for sonication in ringer lactate solution.

Diagnostic. To extract bacteria from the tissue, samples were vortexed using 4mm glass beads (Sarstedt, Nürmbrecht, Germany). After homogenization, samples were directly incubated under aerobic and anaerobic conditions on agar plates and in aerobic thioglycolate broth (BD, Allschwil, Switzerland) for enrichment. The bone samples were inoculated in aerobic thioglycolate broth only. See Table 1 for cultivation time used in this study. For aerobic cultivation Columbia sheep blood agar without antibiotics (BioMérieux, Mary-l'Etoile, France), Colistin nalidixin acid (CNA) blood agar (BioMérieux), MacConkey agar (BioMérieux), and Crowe agar (chocolate agar supplemented with bacitracin and isovitalex (Difco GC medium, Becton Dickinson)) were used. Brucella agar (in house sheep blood agar plates with haemin and vitamin K1, BD), kanamycin-vancomycin agar (laked sheep blood Brucella agar plates with

kanamycin and vancomycin, BD), and phenylethylalcohol agar plates (BD) were used for anaerobic cultivation (Whitley anaerobic workstation MG1000, Don Whitley Scientific, West Yorkshire, England). Thioglycolate broth medium was inspected daily for cloudiness, and then subsequently plated onto chocolate (aerob) and Brucella (anaerob) agar plates for further identification. If thioglycolate broth cultures were negative after 10 days of cultivation, blind subcultures plated on chocolate and Brucella agar plates were performed and cultivated for another 2-3 days.

Explanted implants were directly placed either in a plastic bag (double bagged) or in a sterile tupper ware container in the operating room and transported to the microbiological laboratory for sonication as previously described using BactoSonic (Berlin, Germany) (28, 29). Under a laminar flow, the future opening sites of the bags were thoroughly disinfected using 70% alcohol and the bags were sliced open using a sterile scalpel. The entire fluid inside the inner bag or the tupper ware container was pipetted into a falcon tube (BD) and inoculated onto the corresponding culture media. Of the sonication fluid, 0.5ml was plated onto the distinct agar plates for aerobic (chocolate and sheep blood agar) and anaerobic (Brucella) cultivation as described for tissue before. 10ml of the liquid was inoculated into blood culture flasks (BacT/Alert FA and FN- bottles (Bio-Mérieux) for aerobic and anaerobic cultivation. The bottles were vertically incubated and slightly shaken at 60 hertz. Flasks with a positive growth- signal were subcultured using the set of agar-based media described above. Cultivation times for different methods are summarized in Table 1. A threshold of ≥50 CFU/ml bacteria on direct agar plates was considered as positive.

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All microorganisms were identified by standard identification methods including MALDI-TOF since 2012. Before 2012, identification of *P. acnes* was performed by conventional reactions such as positive reactions for CAMP, indole, nitrate reduction, catalase, and detection of propionic acid as metabolic fatty acids formed by glucose fermentation.

Microscopy

A Gram stain was performed routinely for tissue samples and sonication fluid from implants parallel to all mentioned methods at day zero in the clinical microbiology laboratory.

Time to positivity of P. acnes growth

We defined time to positivity as the time at which either 1) *P. acnes* typical colonies grew on agar plates, 2) when turbidity in thioglycolate broth, or 3) a positive signal in blood culture bottles were detected for which *P. acnes* was finally identified on agar plates.

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RESULTS

Clinical data

We identified 70 cases with a *P. acnes* 'infection', in which 262 out of 379 samples were positive (69.1%). 47 cases did not fulfill our criteria for a proven 'infection' with only one positive sample (47 out of 215 samples, 21.9%). The most common sample site was shoulder (total n = 77), followed by hip (n=26). In the 'infection' group, we diagnosed PJI in 35 (50%) cases, and implant-associated infections with large (plates, intramedullary nail) or small implants (screws, anchors) in 10 (14.3%) and 17 (24.3%), respectively. Five cases presented with septic arthritis (arthroscopy-associated), and three cases with osteomyelitis (two in the shoulder after infiltration or trauma and one in the clavicula associated with b-cell lymphoma). In the 'no infection' group there were pain due to mechanical reasons in 22 (46.8%), aseptic loosening of an implant in 9 (19.2%), and other reasons in 16 (34%) cases diagnosed at revision surgery.

There was no significant difference in number of samples taken between the 'infection' and 'no infection' group (mean 5.4 and 4.6 samples, respectively, p=0.06). The characteristics of cases are shown in Table 2. We found no statistically significant difference with regard to sex, age, or presence of foreign bodies between the two groups. Intraoperative findings described by the surgeon could not help to distinguish between 'infection' and 'no infection'. Isolation of *P. acnes* in the shoulder was more prevalent in the 'infection' group (51 and 26 cases respectively, p=0.073) but isolation in the knee was more frequent in the 'no infection' group (1 and 5 cases respectively, p=0.038).

222 **Time to positivity**

223 The proportion of sample positivity was 55.4% after 7 days and 65.7% after 10 days in
224 the 'infection' group, which was significantly higher than in the 'no infection' group (8.4%
225 and 19.1% respectively, p<0.0001) (Fig. 1).

226 Overall, median time to first positive sample was different in samples of the
227 'infection' group (5 days, 95% CI 4-5) than in the 'no infection' group (9 days, 95% CI 7-
228 10, p<0.0001) (Fig. 2A). Time to confirmation of 'infection', according to our definition
229 equal to time to second positive sample, was also significantly different (6 days, 95% CI
230 5-7) to the single positive sample in the 'no infection' group (9 days, 95% CI 7-10,
231 p<0.0001) (Fig. 2B).

232 Within the 'infection' group, discontinuation of cultures at 7 days would have led
233 to missing diagnosis of 'infection' in 15 out of 70 (21.4%) cases (Fig. 2B). On day 10, a
234 *P. acnes* 'infection' was still not diagnosed in 6 cases (8.6%). These cultures finally
235 turned positive on day 11, all from blind subcultures at the end of regular cultivation of
236 thioglycolate broth. In the 'no infection' group, the majority of cases with a single
237 positive *P. acnes* sample (29 of 47 cases, 61.7%) - suggesting a contamination of the
238 sample - were recorded after day 7 (Fig. 2B).

239 Comparing different cultivation methods (Table 3) in the 'infection' and 'no
240 infection' group, we found that thioglycolate broth of tissue biopsies showed a significant
241 difference in median time to positivity (6 days, 95% CI 5-6 and 9 days, 95% CI 8-10;
242 p<0.0001) as opposed to other methods, which did not significantly differ.

243

Sensitivity, specificity, positive and negative predictive value

Thioglycolate broth as the enrichment method used for tissue and bone biopsies was most effective in correctly identifying *P. acnes* (sensitivity 66.3% in tissue and 75% in bone) and significantly different to aerobic and anaerobic agar plates (sensitivity 5.1% and 42.1% respectively; p-value 0.0001). Low sensitivity was observed in all aerobic cultures (direct aerobic agar or aerobic blood culture bottles) (Table 4). Thioglycolate broth as the method with the highest sensitivity, however, showed a low specificity in tissue (79.1%) and bone (66.7%).

Best specificity with low false-positive results was observed when aerobic agar or aerobic blood culture bottles were used as the enrichment method. Among tissue and sonication fluid cultures, both direct anaerobic cultivation methods showed the best positive predictive value with 96.5% and 86.7% respectively (Table 4).

Gram staining

Only 10 out of 311 (3.2%) samples in the group of patients with a bone and joint 'infection' with an available Gram staining showed a positive result with Gram-positive rods. No positive Gram staining was observed in any of the samples from 'non-infected' individuals resulting in a 100% positive predictive value and 36.9% negative predictive value.

Influence of transportation time on diagnostic output

Transportation time was available in 528 of 594 samples (88.9%) out of which 336 samples were allocated to the 'infection' group and 192 to the 'no infection' group. Transportation time of less than 24 hours was seen in 94% of the positive samples in

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the 'infection' group and in 92% in the 'no infection' group. Transportation time did not correlate with time to positivity (Spearman's rho= -0.0195, p = 0.66).

Sub analysis of 35 cases with a PJI caused by *P. acnes*

In a sub analysis of 35 cases with a PJI caused by *P. acnes*, we compared diagnostic sensitivity of sonication fluid with tissue biopsy cultures. Only 26 cases could be included for this sub analysis with both sonication fluid and tissue biopsy cultures available. 8 cases could not be included due to only having tissue samples and 1 case due to only having tissue and bone samples analyzed.

For tissue biopsies, the sensitivity was 96.2% (25/26 cases) with at least 1 positive culture as opposed to sonication fluid with 46.2% (12/26). 23 cases had ≥2 positive tissue samples, 2 had only 1, and 1 had no positive tissue at all. All 3 cases (11.5%) with < 2 positive samples had a positive sonication >100 cfu/ml to be classified as an 'infection' and would have been misclassified as a contaminant if only tissue biopsies had been cultivated.

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DISCUSSION

In this large cohort-study of 70 *P. acnes* 'infections', we showed that median time to positivity to confirm an 'infection' was 6 days with a range up to 11 days. However, reducing cultivation time to 7 days as proposed by previous studies (20, 23) would have resulted in missing diagnosis in 15 patients (21.4%) suggesting that in patients with a high prevalence of *P. acnes* infections a prolonged cultivation is crucial for detecting infections. This supports the results of a study including fewer *P. acnes* infections (37 cases), which showed that 14% of positive samples were detected after day 7 (3). Furthermore, the study showed that a prolonged cultivation time of > 10 days did not improve sensitivity. Therefore Frangiamore et al. recommended that 10 days of routine cultivation time is optimal to assure accurate diagnosis of *P. acnes* infections. Our data support this recommendation; However, we would recommend a 10-day cultivation with a blind subculture from the thioglycolate at the end in certain cases with high suspicion of *P. acnes* infection. We would have missed 6 cases (8.6%) if we had stopped cultures at day 10.

We reported median time to first positive sample of 5 days. A study by Minassian et al. using blood culture bottles as an enrichment method for tissue biopsies, showed similar results (median time to positivity of 5 days, range 3-13 days) but it was of smaller size with 30 *Propionibacterium* sp. isolates from 16 patients. Butler-Wu et al. described the mean time to positivity of 7.3 ± 2.6 days with a range of up to 13 days for 19 infected cases undergoing revision arthroplasty (18). *P. acnes* grew significantly faster in samples isolated from patients with a *P. acnes* 'infection' as compared to patients without an 'infection'. This difference was most pronounced in enrichment thioglycolate

broth supporting previous studies (3) and our hypothesis that a higher inoculum is to be found in infected samples as compared to contaminated samples.

Thioglycolate broth enrichment during 10 days is a sensitive method to detect *P. acnes.* However, a prolonged incubation time also increases the risk of cultivating a bacterial contamination (30, 31) as shown by our result that 61.7% of samples belonging to our 'no infection' cases were recorded after day 7. Our results are consistent with those of a previous study showing that 21.7% of the cases with only 1 positive *P. acnes* sample - labeled as 'no infection' - became positive after day 13 (18). A previous study published 2013 showed a faster time to positivity when thioglycolate broth was cultivated anaerobically (30) which could be investigated in a prospective study in our orthopedic center. Avoiding the problem of low specificity, we found the best positive predictive value was for tissue biopsies grown directly on anaerobic agar. All aerobic cultivation methods showed low sensitivity, which is not surprising since *P. acnes* is facultative anaerobic. We did not find an increased sensitivity using both aerobic and anaerobic cultures as Butler-Wu et al. published (18).

Gram staining is not a useful tool for excluding bone and joint infections. All of our 10 positive Gram stains were from 'infected' cases. This supports published data showing that positive results prove infection while a negative result does not exclude an infection (32, 33).

Cultivation of sonication fluid of implants is known to show a better sensitivity than conventional tissue cultures (27). This is also shown in a recent study by Portillo et al. who investigated sensitivity of sonication in 39 orthopedic implant-associated infections including 5 cases with a *P. acnes* infection. They detected all 5 *P. acnes*

infections by sonication but only 2 by conventional tissue cultures (22). A retrospective cohort study of 20 PJIs caused by *P. acnes* showed an 89% sensitivity for sonication fluid cultures and 60% for conventional tissue cultures (15). Our results of lower sensitivity in sonication than in tissue cultures refute this observation: The most obvious explanation for our findings is our short cultivation time of only 3 days on agar plates and 7 days in blood culture bottles compared to a longer cultivation time in tissue or bone biopsy cultures. Other studies using cultivation times of up to 14 days in blood culture bottles reported a high sensitivity for sonication (21, 22, 24). An alternative explanation for our low sensitivity with sonication is the possibility that *P. acnes* might be inhibited or killed using ultrasound baths as has been shown for other pathogens (34). Since we had initially used plastic bags instead of tupper ware containers for sonication for many years (using a higher ultrasound frequency of 45kHz), this could contribute to the difference and further emphasizes the necessity to adhere to a standard protocol. Our results of better sensitivity of direct agar planting for 2-3 days compared to cultivation using blood culture bottles for 7 days is intriguing particularly in light of the increasing number of studies that point to improved performance of blood culture media for the recovery of pathogens causing PJI (21, 22, 24). An advantage of using blood culture bottles instead of thioglycolate broth medium with presence of antibiotic absorbing agents could not be assessed at this point since we excluded patients taking antibiotics.

We showed that a longer transportation time from the operating theatre to the microbiology laboratory did not influence time to positivity of *P. acnes* growth. This is in contrast to recent IDSA recommendation (35) emphasizing that anaerobic tissue

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cultures should be transported at room temperature in anaerobic containers as soon as possible with an optimal time of 2 hours. However, our result supports the hypothesis that microorganism in the biofilm state as found in bone and joint infections are robust to environmental changes in particular *P. acnes* as a facultative anaerobic bacterium. We could not find another study investigating the influence of time of transportation or use of anaerobic transport container for recovery of *P. acnes* in bone and joint infections.

The strength of our study is the large cohort of 70 cases with a *P. acnes* infection. To our knowledge, this is the largest study to date with analysis of the applied cultivation methods and time to positivity in bone and joint infections focusing on *P. acnes* as a slow growing microorganism. Most study results in this field are influenced by various variables. To strengthen the conclusions of our analysis, we excluded samples obtained from individuals already on antibiotics as well as cases with a polymicrobial infection. The Strength of our study is the time period of over 10 years in which the same microbiological protocols were used for all bone and joint infection samples. A limitation of our study is that different culture media were not incubated for the same period of time, which makes comparison difficult but due to the retrospective study design difficult to correct.

We conclude that a prolonged cultivation time of 10 days is necessary for *P. acnes* identification. We do not recommend reducing cultivation time to 7 days in patient cohorts with a high incidence of *P. acnes* infections (e.g shoulder PJI, vertebral osteomyelitis). We would have missed 21.4% of the *P. acnes* infections if cultivation time had been reduced to 7 days. Thioglycolate broth as an enrichment method in tissue biopsies showed a high sensitivity. The best positive predictive value was seen

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with direct incubation on anaerobic agar plates. Time to positivity of *P. acnes* growth does not seem to be affected by a prolonged transportation time, which shows that *P. acnes* in the biofilm of musculoskeletal infections can survive for hours even in a fastidious environment.

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499 # TABLES AND FIGURES

500 **Table 1.** Standard cultivation times for periimplant-tissue, implant (sonication fluid), and
501 bone and accepted minimum cultivation time to be included in our study.

	Cultivation method	Standard cultivation time (days)	Accepted cultivation time for inclusion (days)
Tissue	Direct aerobic	7	≥6
	Direct anaerobic	7	≥6
	Thioglycolate aerobic	10 (-14*)	≥9
Implant	Direct aerobic	2-3	≥2
	Direct anaerobic	2-3	≥2
	Aerobic BCB	7	≥6
	Anaerobic BCB	7	≥6
Bone	Thioglycolate aerobic	10 (-14*)	≥9

502 *prolonged culture with blind subculture at the end
503 BCB, blood culture bottles
504

505 **Table 2.** Characteristics of 70 cases with a bone and joint 'infection' (≥ 2 positives *P.*
506 *acnes* samples) and 47 cases with 'no infection' (1 positive *P. acnes* culture).

	'Infection' (n =70)	'No infection' (n =47)	*p*-value
Patient characteristics			
Sex, female (%)	21 (30%)	15 (32%)	0.84
Age years, median (range)	58 (21-81)	58 (16-86)	0.49
Sample site n (%)			
Shoulder	51 (72.9%)	26 (55.3%)	0.07
Hip	13 (18.6%)	13 (27.7%)	0.27
Spine	4 (5.7%)	1 (2.1%)	0.65
Knee	1 (1.4%)	5 (10.6%)	0.04
Other	1 (1.4%)	2 (4.3%)	0.56
Presence of			
any foreign body n (%)	62 (88.6%)	39 (83%)	0.42
prosthesis n (%)	35 (50%)	27 (57.4%)	0.46
Intraoperative presentation			
Normal	7 (10%)	6 (12.8%)	0.77
Pus	9 (12.9%)	2 (4.3%)	0.20
Inflammation	41 (58.6%)	26 (55.3%)	0.85
Wear of the implant	1 (1.4%)	2 (4.3%)	0.56
Adhesion	10 (14.3%)	7 (14.9%)	1

	'Infection' (n =70)	'No infection' (n =47)	*p*-value
No data available	2 (2.8%)	4 (8.5%)	0.22

Nr, number; SD, standard deviation

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509 **Table 3.** Median time to positivity (TtP) for different cultivation methods in the 'infection'
510 and 'no infection' group.

Culture type	Culture method	'Infection'		'No infection'	
		Positive cultures (Nr)	TtP, days (median, 95% CI)	Positive cultures (Nr)	TtP, days (median, 95% CI)
Tissue	Direct aerobic	15	7 (5-7)	2	7
	Direct anaerobic	136	6 (6-6)	5	6
	Thioglycolate aerobic	205	6 (5-6)	38	9 (8-10)
Implant	Direct aerobic		-		-
	Direct anaerobic	13	4	2	3
	Aerobic BCB		-		-
	Anaerobic BCB	4	4	1	5
Bone	Thioglycolate aerobic	6	7	2	8

511 Nr, number; BCB, blood culture bottles; CI, confidence interval; TdP, Time to Positivity

512 **Table 4**. Sensitivity, specificity, positive predictive value (PPV), and negative predictive
513 value (NPV) of different culture methods

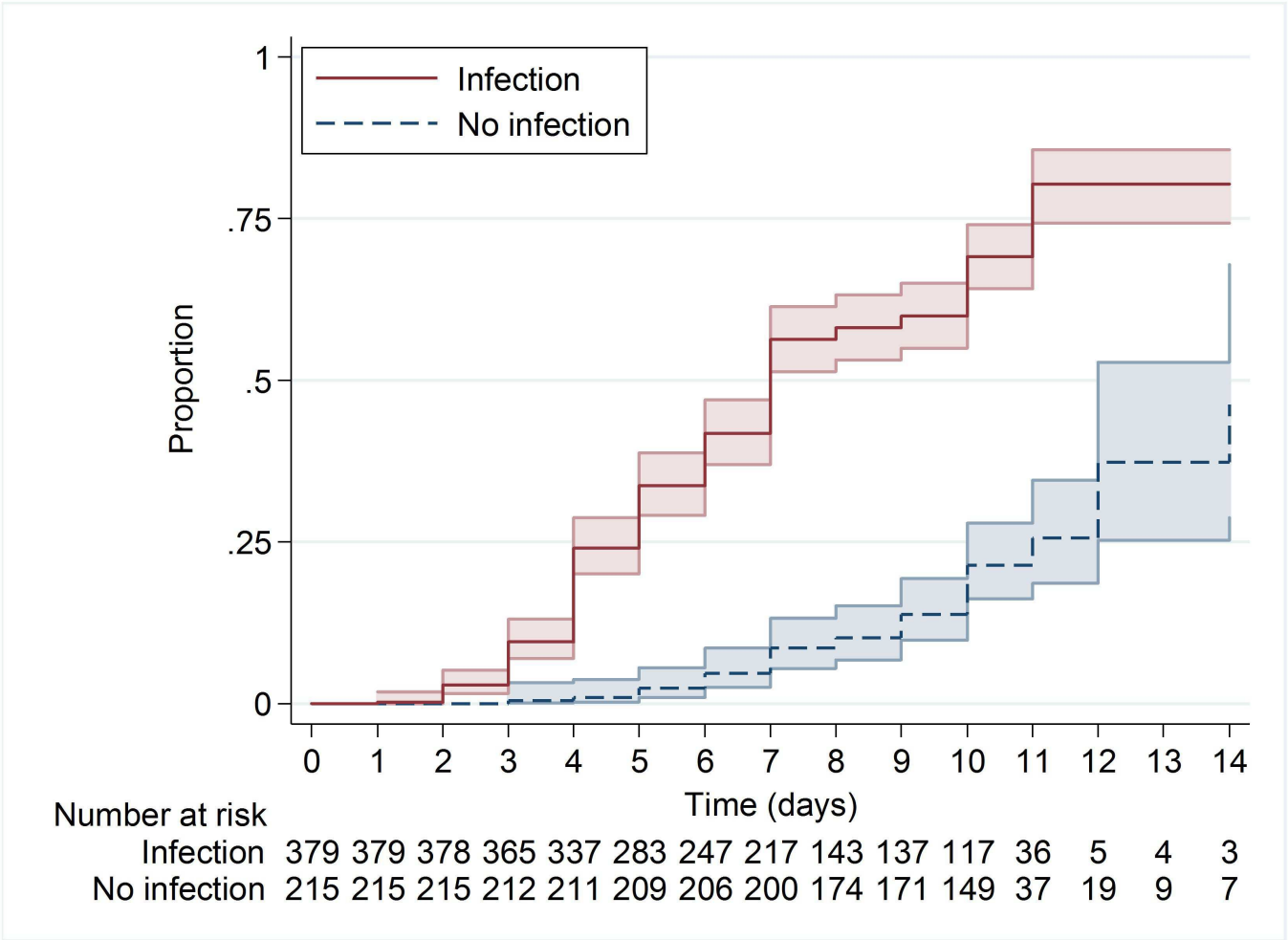
	Culture method	Sensitivity	Specificity	PPV	NPV
Tissue	Direct aerobic	5.1%	98.9%	88.2%	38.8%
	Direct anaerobic	42.1%	97.1%	96.5%	47.3%
	Thioglycolate aerobic	66.3%	79.1%	84.4%	58.1%
Implant	Direct aerobic	0%	100%	0%	36.4%
	Direct anaerobic	37.1%	91.3%	86.7%	48.8%
	Aerobic BCB	0%	100%	0%	38.3%
	Anaerobic BCB	13.3%	95.2%	80.0%	43.5%
Bone	Thioglycolate aerobic	75.0%	66.7%	75.0%	66.7%

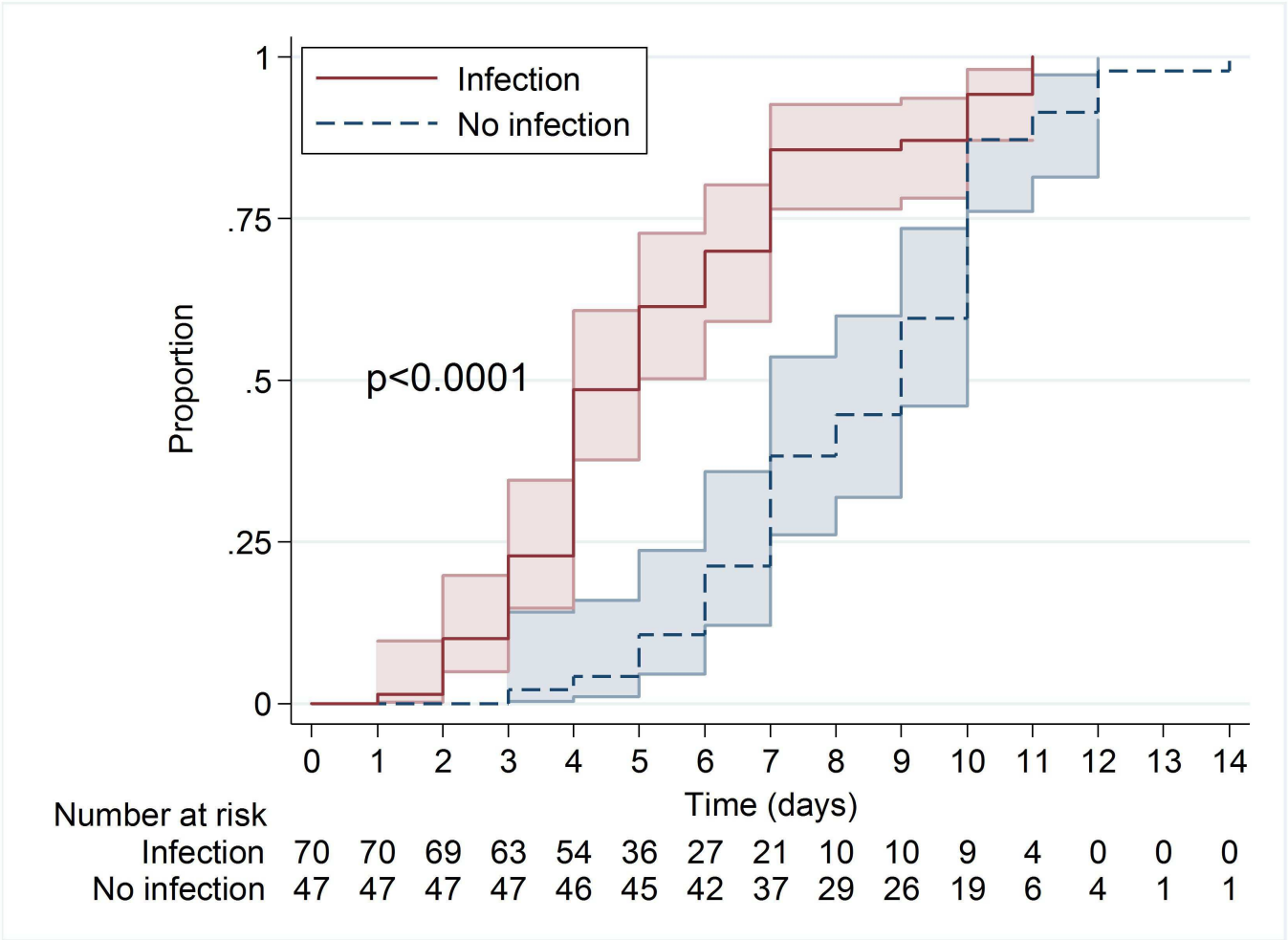
514 PPV, positive predictive value; NPV, negative predictive value
515 BCB, blood culture bottles

FIGURE LEGENDS

Fig. 1. Proportion of sample positivity with *P. acnes* in the 'infection' and 'no infection' group. The colored areas represent the 95% confidence interval. The proportion of positivity at day 7 was 55.4% and 65.7% at day 10 in the 'infection' group. In the 'no infection' group only 8.4% were positive at day 7, and 19.1% at day 10 ($p<0.0001$). Discontinuation of sample cultivation would have resulted in missing 52 of 262 (19.9%) positive samples or 15 of 70 (21.4%) cases.

Fig. 2. Proportion of first (A) or second (confirmation) (B) positive sample in the 'infection' group compared to the single positive sample in the 'no infection' group. The colored areas represent the 95% confidence interval. The median time to positivity was 5 (95% CI 4-5) days for the first sample, 6 (95% CI 5-7) days for the second positive sample in the 'infection' group to confirm 'infection', and 9 (95% CI 7-10) days in the 'no infection' group.





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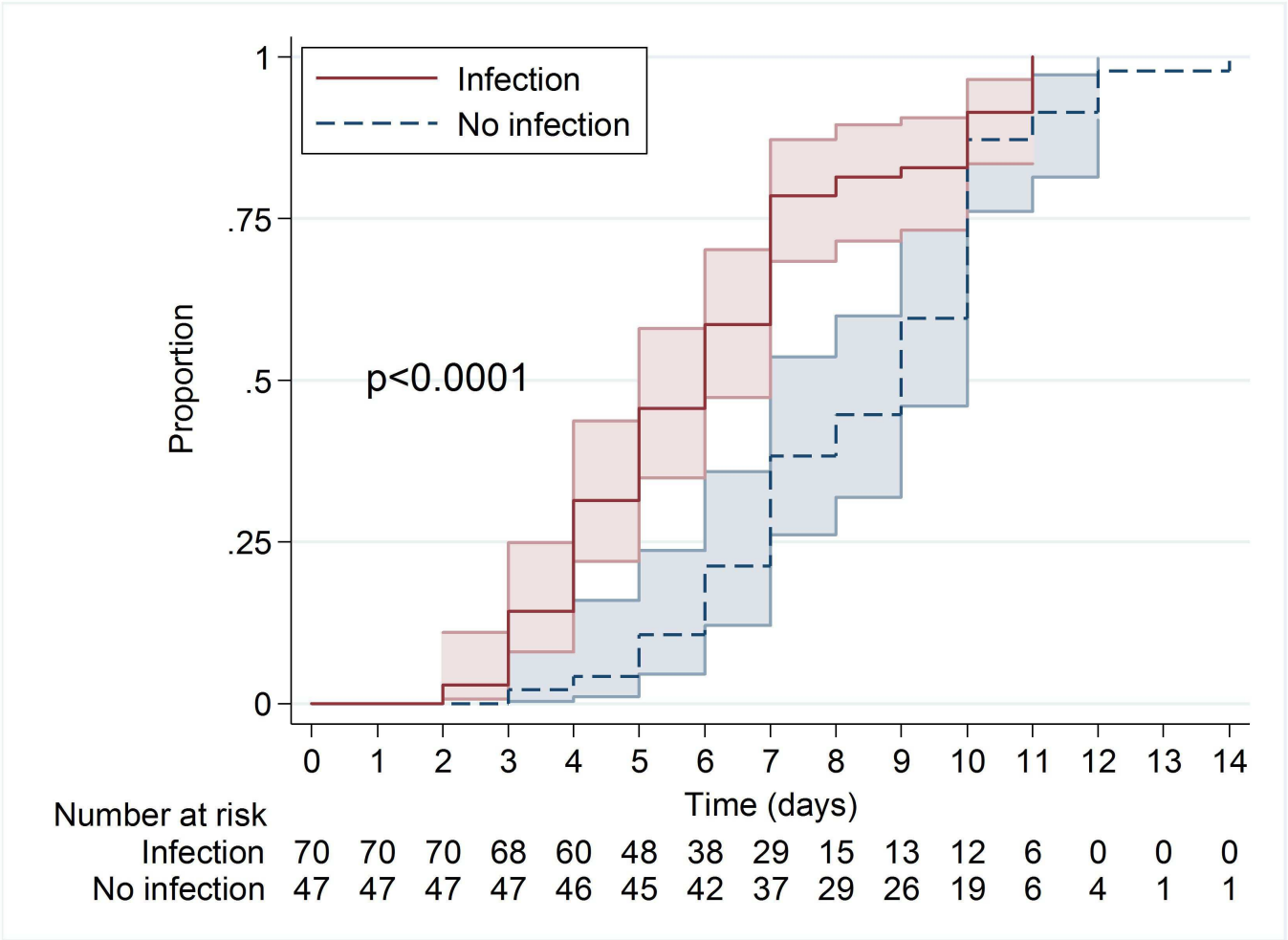

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